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Tumor necrosis factor receptors 1 and 2 are associated with early glomerular lesions in type 2 diabetes

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Abstract

Elevated serum tumor necrosis factor receptor 1 (TNFR1) and 2 (TNFR2) concentrations are strongly associated with increased risk of end-stage renal disease in type 2 diabetes. However, little is known about the early glomerular structural lesions that develop in patients when these markers are elevated. Here we examined the relationships between TNFRs and glomerular structure in 83 American Indians with type 2 diabetes. Serum TNFRs and glomerular filtration rates (GFR, iothalamate) were measured during a research exam performed within a median of 0.9 months from a percutaneous kidney biopsy. Associations of TNFRs with glomerular structural variables were quantified by Spearman's correlations and by multivariable linear regression after adjustment for age, gender, diabetes duration, hemoglobin A1c, body mass index, and mean arterial pressure. The baseline mean age was 46 years, median GFR 130 ml/min, median albumin/creatinine ratio 26 mg/g, median TNFR1 1500 pg/ml, and median TNFR2 3284 pg/ml. After multivariable adjustment, TNFR1 and TNFR2 significantly correlated inversely with the percentage of endothelial cell fenestration and the total filtration surface per glomerulus. There were significant positive correlations with mesangial fractional volume glomerular basement membrane width, podocyte foot process width, and percent of global glomerular sclerosis. Thus, TNFRs may be involved in the pathogenesis of early glomerular lesions in diabetic nephropathy.

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Keywords

diabetic nephropathy; endothelium; kidney biopsy

Introduction

Serum tumor necrosis factor receptor 1 (TNFR1), and receptor 2 (TNFR2) concentrations are strong independent predictors of renal function decline leading to end-stage renal disease (ESRD) in Caucasians and American Indians with diabetes.¹⁻⁴

Although *in vitro* activated TNFR1 induces tissue injury through proinflammatory signals and/or cell death and TNFR2 promotes cell migration, regeneration and proliferation, and regulates TNFR1 induced apoptosis,⁵ very little is known about the early glomerular structural lesions in kidneys that develop in humans when these markers are elevated. Further, since TNF α binds to the TNFRs, it is not known whether these early glomerular lesions are associated with the serum concentration of TNF α or with the TNFRs. In one small study, TNFR1, but not TNFR2, was associated with higher mesangial fractional volume in 22 persons with type 2 diabetes.⁶

In the present study, we examined the relationships between serum concentrations of TNF α , TNFR1, and TNFR2 and glomerular lesions in American Indians with type 2 diabetes and normal or elevated renal function. The glomerular morphometric data were obtained from a kidney biopsy performed at the end of a 6-year randomized clinical trial that evaluated the renoprotective efficacy of the angiotensin receptor blocker losartan in diabetic nephropathy.⁷ The TNF markers were measured in serum obtained at a research examination coincident with the biopsy. The TNF markers that demonstrated univariate associations with glomerular structural lesions were further confirmed using multivariable models.

Results

Clinical and demographic characteristics of the study participants are shown in Table 1. The study included 83 participants with type 2 diabetes (63 female, 20 male), with a mean age of 46 \pm 10 years. Forty three (52%) had an albumin-to-creatinine ratio (ACR) <30 mg/g, 24 (29%) had moderate albuminuria (30 to 299 mg/g), and 16 (19%) had severe albuminuria (\geq 300 mg/g). Seventy two (86%) had measured glomerular filtration rate (mGFR, iothalamate) above 90 ml/min and 81 (98%) had mGFR above 60 ml/min. When mGFR was standardized to body surface area, 66 (79%) had mGFR above 90 ml/min/1.73 m² and 78 (94%) were above 60 ml/min/1.73 m². Hyperfiltration, defined by an mGFR \geq 154 ml/min, a value two standard deviations above the mean mGFR for Pima Indians with normal glucose tolerance, was present in 29 individuals (35%).

Serum concentrations of free TNF α and the TNFRs were measured in samples collected at a research examination closest to the kidney biopsy (median of 0.9 months apart, interquartile range [IQR]=0.8-1.9 months). Accordingly, 70 participants (84%) were still enrolled in the clinical trial. Thirty nine (47%) of the participants were assigned to the placebo group and 44 (53%) were assigned to the losartan treatment group during the clinical trial. Table 2

shows the distribution of measured biomarkers and other clinical characteristics at that research examination by the 25th and 75th percentiles of TNFR1 and TNFR2. The mGFR was lower (but not significantly so for TNFR2) and ACR was higher with higher concentrations of either TNFR. Enrollment in the treatment arm of the clinical trial was more common among those in lower percentile groupings of TNFR1 and TNFR2, but not significantly so. Several glomerular structural variables were significantly associated with percentiles of TNFR1 and TNFR2, as shown in Table 3. Mesangial fractional volume and podocyte foot process width were higher with higher TNFR1 and TNFR2 concentrations, whereas total filtration surface per glomerulus, and percent of the capillary endothelial cell surface covered with normal fenestrations were lower.

TNFR1 and TNFR2 correlated positively with each other ($r=0.84$, $p<0.001$) and with ACR ($r=0.36$ and 0.37 , respectively; $p<0.001$ for each correlation), and inversely with mGFR ($r=-0.35$, $p=0.001$; $r=-0.28$, $p=0.01$) (Table 4). Neither TNFR correlated significantly with TNF α . Higher TNFR1 and TNFR2 correlated inversely with percentage of normally fenestrated endothelium ($r=-0.42$ and -0.43 ; $p<0.001$ for each correlation), total filtration surface per glomerulus ($r=-0.27$, $p=0.01$; $r=-0.29$, $p=0.007$), and filtration slit frequency ($r=-0.24$, $p=0.03$; $r=-0.29$, $p=0.008$), and positively with mesangial fractional volume ($r=0.36$ and 0.38 , $p<0.001$ for both correlations), glomerular basement membrane width ($r=0.23$, $p=0.04$; $r=0.26$, $p=0.02$), and podocyte foot process width ($r=0.29$, $p=0.007$; $r=0.31$, $p=0.004$). TNFR1, but not TNFR2, correlated positively with percent global glomerular sclerosis ($r=0.25$, $p=0.02$) and inversely with the number of podocytes per glomerulus ($r=-0.23$, $p=0.04$). TNF α correlated positively with A1c ($r=0.27$, $p=0.02$) and MAP ($r=0.23$, $p=0.048$), but it had no significant univariate correlations with any glomerular variables.

Given the significant univariate associations of the TNFRs with glomerular lesions, these relationships were then examined after adjusting for age, sex, diabetes duration, A1c, body mass index, and mean arterial pressure (Model 1). Both TNFRs remained significantly and inversely correlated with the total filtration surface per glomerulus (TNFR1 partial $r=-0.25$, $p=0.03$; TNFR2 partial $r=-0.28$, $p=0.009$) and percentage of normally fenestrated endothelium (TNFR1 partial $r=-0.41$, $p=0.001$; TNFR2 partial $r=-0.37$, $p=0.0006$) and positively with mesangial fractional volume (TNFR1 partial $r=0.44$, $p<0.001$; TNFR2 partial $r=0.38$, $p=0.0005$), glomerular basement membrane width (TNFR1 partial $r=0.34$, $p=0.002$; TNFR2 partial $r=0.30$, $p=0.006$), percent global glomerular sclerosis (TNFR1 partial $r=0.22$, $p=0.04$; TNFR2 partial $r=0.23$, $p=0.04$), and podocyte foot process width (TNFR1 partial $r=0.30$, $p=0.006$; TNFR2 partial $r=0.25$, $p=0.02$). Figure 1 shows the strongest of these correlations. The associations between TNFRs and morphometric variables remained unchanged after including treatment assignment from the clinical trial in the multivariable linear regression models (Model 2, Figure 2). When interaction terms between the TNFRs and treatment assignment were added to these models, most were not significant, indicating that the relationship between the TNFRs and most morphometric variables was not modified by treatment. Treatment assignment did modify the relationship between each TNFR and foot process width ($p=0.02$ for TNFR1, $p=0.02$ for TNFR2) and filtration slit frequency ($p=0.001$ for TNFR1, $p=0.001$ for TNFR2).

Adding ACR and mGFR to the regression models (Model 3) reduced the associations between TNFRs and glomerular lesions, but associations remained significant for mesangial fractional volume (TNFR1 partial $r=0.27$, $p=0.01$; TNFR2 partial $r=0.26$, $p=0.02$) and percentage of normally fenestrated endothelium (TNFR1 partial $r=-0.29$, $p=0.008$; TNFR2 partial $r=-0.25$, $p=0.02$) in the full model (Figure 2). Treatment assignment modified the relationship between each TNFR and foot process width ($p=0.02$ for TNFR1, $p=0.02$ for TNFR2) and filtration slit frequency ($p=0.002$ for TNFR1, $p=0.001$ for TNFR2) in these models as well. For illustration, Figure 3 shows electron micrographs of glomerular endothelium that is either normally fenestrated or has reduced fenestrations. Supplemental Figures 1 and 2 show the un-cropped images with scale bars.

When models were examined separately by treatment assignment, the power to detect associations between TNFR1 or TNFR2 and the morphometric variables was reduced, but the direction of association remained the same in the two subgroups. Furthermore, the morphometric variables associated with the TNFRs in the whole group remained associated in one or both subgroups (Supplemental Table 1).

Discussion

In the present study, we found that elevated serum concentrations of TNFR1 or TNFR2 in patients with type 2 diabetes are associated with early glomerular structural lesions. These receptors showed the strongest associations with reduced percentage of normally fenestrated endothelium and with increased mesangial fractional volume after controlling for the effects of relevant clinical covariates. Weaker associations were found with glomerular basement membrane width, podocyte foot process width, and with total filtration surface per glomerulus. These findings provide evidence that elevated serum concentrations of TNFRs coincide with early lesions in specialized filtration structures in the kidneys of persons with type 2 diabetes. Since we found no correlations between free TNF α and the glomerular structural lesions, we postulate that the TNFRs, and not TNF α , are the major players in the disease process that leads to such early structural lesions.

In previous studies, elevated serum TNFRs,^{8,16} increased mesangial fractional volume,^{17,19} and reduced endothelial cell fenestrations associated with elevated albuminuria and low GFR,^{20,21} but the present study is the first to show correlations between serum TNFR1 and TNFR2 and glomerular lesions in patients with type 2 diabetes.

The fenestrated endothelium, covered by the endothelial glycocalyx, represents the intraluminal barrier to the passage of macromolecules, including albumin, across the glomerular capillary. Normally, fenestrations occupy 30-50% of the glomerular endocapillary area and are densely distributed opposite the podocyte foot processes to facilitate filtration.^{22,23} Loss of endothelial cell fenestrations in glomerular capillaries reflects generalized microvascular disease due to persistent hyperglycemia, and is associated with low-level albuminuria.^{24,25} This endothelial dysfunction also appears to contribute to podocyte injury and loss of GFR through several mechanisms.²⁶ In a subset of the present cohort, we reported previously that the frequency of normally fenestrated endothelium in normoalbuminuric patients with type 2 diabetes was significantly lower than in healthy

kidney donors (27.4% and 43.5%, respectively) and worsened with higher albuminuria.²⁰ The present study confirms these relationships in a larger cohort of participants with type 2 diabetes, and it also identifies robust and independent inverse correlations between overall density of endothelial cell fenestrations and circulating TNFRs. These associations suggest that a TNFR-related pathway may link early structural lesions of the glomerular endothelium with progression of renal disease in the setting of diabetes.

Mesangial expansion is a structural hallmark of diabetic nephropathy and correlates strongly with GFR reduction.²⁷ In the present study, TNFRs remained associated with mesangial fractional volume after controlling for the effects of traditional risk factors. This finding replicates results of a previous cross-sectional study of 22 patients with type 2 diabetes (9 with normoalbuminuria, 13 with moderate albuminuria)⁶ which found that TNFR1 correlates with mesangial expansion after controlling for age, body mass index, and blood pressure, whereas TNFR2 did not correlate with either mesangial fractional volume or cortical interstitial fractional volume, the only two morphometric characteristics included in the analysis.

Although the present study describes only cross-sectional associations, a number of earlier longitudinal studies have established that elevated levels of circulating TNFRs are powerful predictors of renal function decline and ESRD. Indeed, a previous study in Pima Indians with type 2 diabetes and preserved kidney function, found that higher serum concentrations of TNFR1 or TNFR2 were associated with 60% to 70% higher risk of progression to ESRD during a median follow-up of 9.5 years.⁴ Both receptors enhanced the discriminatory power of clinically recognized risk factors for kidney disease progression. These findings are consistent with our original report from the Joslin Clinic cohort in which we showed a very robust association between serum concentrations of TNFRs and the risk of ESRD in Caucasians with type 2 diabetes who were followed for 8-12 years.¹ We also found in patients from the Joslin Clinic cohort who had type 1 diabetes, normal or moderate albuminuria, and normal kidney function at baseline, that elevated TNFRs predicted estimated GFR <60 ml/min per 1.73 m².² Our present study is also consistent with previous reports in which the risks of ESRD or impaired renal function were independent of serum free or total TNF α concentrations.^{1,2} Among patients with type 1 diabetes who were enrolled in the Diabetes Control and Complications Trial (DCCT) and had normal albuminuria and no cardiovascular disease at baseline, increased serum TNFR1 and TNFR2 were associated with 30-50% higher odds of severe albuminuria.²⁸ However, the risk of impaired renal function according to the baseline concentrations of TNFRs was not reported in the DCCT cohort.

Strengths of the present study include a phenotypically well-characterized population with kidney disease attributable nearly exclusively to diabetes and in which earlier detection of diabetes allows the initial stages of diabetic nephropathy to be studied.²⁹ A limitation is the cross-sectional design which precludes causal inferences about the associations between circulating TNFRs and glomerular morphologic changes. Nevertheless, multiple previous prospective studies consistently demonstrate that high serum concentrations of TNFRs strongly predict the renal function decline that leads to ESRD, and we now show that TNFRs are associated with the principal glomerular structural antecedents of this decline.

Another potential limitation is that the study was conducted in the context of a clinical trial in which the treatment being evaluated was associated with structural preservation among participants with moderate albuminuria. Nevertheless, the conclusions were unchanged when we accounted for treatment assignment in the analysis. We also presented results separately by treatment assignment, and they are generally consistent with the results from the entire cohort (Supplemental Table 1). Each analyte was measured only once, but we reported previously that TNFR1 and TNFR2 concentrations are stable over 2-3 years.²

In summary, elevated serum concentrations of TNFR1 or TNFR2 correlate with glomerular structural lesions in patients with type 2 diabetes and normal or high-normal GFR. The most prominent correlations are with the percentage of normally fenestrated endothelium and with the mesangial fractional volume, after adjusting for the effects of traditional risk factors. These findings suggest that the TNFRs may be involved in the pathogenesis of early glomerular lesions in diabetic nephropathy.

Materials and Methods

Study Participants

Between 1965 and 2007, Pima Indians from the Gila River Indian Community participated in a longitudinal study of diabetes and its complications. Each member of this community who was at least 5 years old was invited to have a research examination approximately every 2 years. Diabetes was diagnosed by a 2-hour post-load plasma glucose concentration ≥ 200 mg/dl (11.1 mmol/l) at these biennial examinations, or when the diagnosis was otherwise documented in the medical record. For the present study, we selected participants from this longitudinal population based study who had type 2 diabetes and also participated in a 6-year randomized, placebo-controlled clinical trial to evaluate the renoprotective efficacy of losartan ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00340678) number NCT00340678).⁷ By design, participants in the clinical trial were stratified at baseline into two groups according to the level of urinary albumin excretion during screening, and they were randomized to receive either placebo or losartan within each group. In one stratum, participants had normal ACR and in the other they had moderate ACR, so the study included participants who were clinically normal. The clinical trial included annual measurements of GFR by the urinary clearance of iothalamate and a kidney biopsy at the end of the treatment period. Treatment was associated with preservation of mesangial fractional volume among participants with moderate albuminuria at baseline. Of the 111 participants who had morphometric measurements as part of the clinical trial, 83 who had available serum to measure the TNFRs at the research examination closest to the kidney biopsy were included in the present study. Forty-four participants (53%) were in the normal ACR stratum at the onset of the clinical trial. TNF α was also measured in a subset of 74 participants who had sufficient remaining serum volume to do so. This study was approved by the Institutional Review Board of the National Institute of Diabetes and Digestive and Kidney Diseases. Each subject gave informed consent.

Laboratory Measurements

Laboratory measurements from the research examination closest to the kidney biopsy are reported. All urine and serum samples were stored at -80°C until assay. Urinary albumin was

measured by nephelometric immunoassay and urinary creatinine by a modified Jaffé reaction.^{30,31} Urinary albumin concentrations below the threshold detected by the assay (<6.8 mg/L) were set to 6.8 mg/L in the analyses. Urinary albumin excretion was estimated by computing the urinary ACR in units of mg/g. A1c was measured by high-performance liquid chromatography. A high-performance liquid chromatography system also was used to measure urinary clearance of non-radioactive iothalamate for GFR determination.³²

Serum concentrations of free TNF α and the TNFRs were measured by ELISA in Dr. A. Krolewski's laboratory, Joslin Diabetes Center, Boston, MA, according to the same protocol used in the Joslin Kidney Study.¹ Intra-assay coefficient of variation (CV) for mGFR was 1.1%, for TNFR1 and TNFR2 were <5%, and for TNF α <10%; the inter-assay CVs were 2.9%, 16%, 5%, and 15.8%, respectively. Reproducibility of the TNFR assays was assessed by intra-class correlation of measurements from 21 duplicate samples blinded to the performance laboratory. The intra-class correlation for TNFR1 was 0.92 and for TNFR2 was 0.99, reflecting good agreement.

Body mass index was defined as weight divided by the square of height (kg/m²). Mean arterial pressure was calculated as MAP = 2/3 diastolic arterial pressure + 1/3 systolic arterial pressure.

Morphometric Methods

Masked unbiased random sampling morphometric methods were used to measure structural parameters.⁷ Digital light and electron micrographs were measured using formal stereologic methods to account for two-dimensional sampling of three-dimensional objects.³³

Morphometric variables examined included mean glomerular volume, percent globally sclerotic glomeruli, cortical interstitial fractional volume, mesangial fractional volume, total filtration surface per glomerulus, GBM width, number of endothelial cells+mesangial cells per glomerulus, number of podocytes per glomerulus, filtration slit frequency, podocyte foot process width, percent podocyte detachment, and percentage of normally fenestrated endothelium.^{7,20,21,28,34} An equation that accounts for the smaller diameter of sclerotic glomeruli, and the consequent difference in the probability of encountering a sclerotic or nonsclerotic glomerulus in a random cross-section, was used to calculate the percentage of sclerotic glomeruli.³⁵ An average of 15 \pm 6 glomeruli were examined in each participant by light microscopy and 3 \pm 1 by electron microscopy for the morphometric measurements. Glomerular variables for each individual were calculated as the mean of all glomeruli evaluated for that individual.

Statistical Analyses

Clinical and demographic features are presented as medians and IQRs or as means and standard deviations (SDs) in all participants combined and in three groups of participants separated at the 25th and 75th percentiles of TNFR1. The Kruskal-Wallis test was used to compare variables across percentiles of TNFR1; Mantel-Haenszel χ^2 tests for general association were used to examine relationships between TNFR percentiles and the categorical variables hypertension, lipid lowering, and hypoglycemic treatment. Associations between clinical characteristics, glomerular structural variables, and the

biomarkers were explored by Spearman's correlation. Partial correlation analysis was used to study the relationships between biomarkers and structural measurements after adjustment for the effects of age, sex, diabetes duration, blood pressure, body mass index, and A1c by multivariable linear regression (Model 1). Associations between biomarkers and morphometric measures were assessed graphically using the partial regression residual plot (*i.e.*, plot of the correlation between the residuals from regressing the biomarkers on covariates described above and the residuals from regressing morphometric measurements on covariates).

Two sensitivity analyses were performed. In the first analysis, treatment assignment from the clinical trial was added to the linear regression models (Model 2). In the second, we added mGFR and ACR to the linear regression models (Model 3).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Niewczas MA, Gohda T, Skupien J, et al. Circulating TNF receptors 1 and 2 predict ESRD in type 2 diabetes. *J Am Soc Nephrol.* 2012; 23:507–515. [PubMed: 22266663]
2. Gohda T, Niewczas MA, Ficociello LH, et al. Circulating TNF receptors 1 and 2 predict stage 3 CKD in type 1 diabetes. *J Am Soc Nephrol.* 2012; 23:516–524. [PubMed: 22266664]
3. Forsblom C, Moran J, Harjutsalo V, et al. FinnDiane Study Group Added value of soluble tumor necrosis factor- α receptor 1 as a biomarker of ESRD risk in patients with type 1 diabetes. *Diabetes Care.* 2014; 37:2334–2342. [PubMed: 24879837]
4. Pavkov ME, Nelson RG, Knowler WC, et al. Elevation of circulating TNF receptors 1 and 2 increases risk of end-stage renal disease in American Indians with type 2 diabetes. *Kidney Int.* 2015; 87:812–819. [PubMed: 25272234]
5. Locksley RM, Killeen N, Lenardo MJ. The TNF and TNF receptor superfamilies: integrating mammalian biology. *Cell.* 2001; 104:487–501. [PubMed: 11239407]
6. Fernández-Real JM, Vendrell J, García I, et al. Structural damage in diabetic nephropathy is associated with TNF- α system activity. *Acta Diabetol.* 2012; 49:301–305. [PubMed: 22042131]
7. Weil EJ, Fufaa G, Jones LI, et al. Effect of Losartan on prevention and progression of early diabetic nephropathy in American Indians with type 2 diabetes. *Diabetes.* 2013; 62:3224–3231. [PubMed: 23545707]
8. Vendrell J, Broch M, Fernandez-Real JM, et al. Tumor necrosis factor receptors (TNFRs) in Type 2 diabetes. Analysis of soluble plasma fractions and genetic variations of TNFR2 gene in a case–control study. *Diabet Med.* 2005; 22:387–392. [PubMed: 15787661]
9. Lin J, Hu FB, Rimm EB, et al. The association of serum lipids and inflammatory biomarkers with renal function in men with type II diabetes mellitus. *Kidney Int.* 2006; 69:336–342. [PubMed: 16408124]

10. Ng DP, Fukushima M, Tai BC, et al. Reduced GFR and albuminuria in Chinese type 2 diabetes mellitus patients are both independently associated with activation of the TNF- α system. *Diabetologia*. 2008; 51:2318–2324. [PubMed: 18839132]
11. Niewczas MA, Ficociello LH, Johnson AC, et al. Serum concentrations of markers of TNF α and Fas-mediated pathways and renal function in nonproteinuric patients with type 1 diabetes. *Clin J Am Soc Nephrol*. 2009; 4:62–70. [PubMed: 19073786]
12. Krolewski AS, Niewczas MA, Skupien J, et al. Early progressive renal decline precedes the onset of microalbuminuria and its progression to macroalbuminuria. *Diabetes Care*. 2014; 37:226–234. [PubMed: 23939543]
13. Saulnier PJ, Gand E, Ragot S, et al. Association of serum concentration of TNFR1 with all-cause mortality in patients with type 2 diabetes and chronic kidney disease: follow-up of the SURDIAGENE Cohort. *Diabetes Care*. 2014; 37:1425–1431. [PubMed: 24623026]
14. Lin J, Hu FB, Mantzoros C, et al. Lipid and inflammatory biomarkers and kidney function decline in type 2 diabetes. *Diabetologia*. 2010; 53:263–267. [PubMed: 19921505]
15. Skupien J, Warram JH, Niewczas MA, et al. Synergism between circulating tumor necrosis factor receptor 2 and HbA(1c) in determining renal decline during 5-18 years of follow-up in patients with type 1 diabetes and proteinuria. *Diabetes Care*. 2014; 37:2601–2608. [PubMed: 24898299]
16. Izumi Y, Yabe D, Taniguchi A, et al. Circulating TNF receptor 2 is associated with the development of chronic kidney disease in non-obese Japanese patients with type 2 diabetes. *Diabetes Res Clin Pract*. 2013; 99:145–150. [PubMed: 23375231]
17. White KE, Bilous RW. for The Collaborative Study Group. Type 2 diabetic patients with nephropathy show structural–functional relationships that are similar to type 1 disease. *J Am Soc Nephrol*. 2000; 11:1667–1673. [PubMed: 10966491]
18. Mauer M, Drummond K. for the International Diabetic Nephropathy Study Group. The early natural history of nephropathy in type 1 diabetes I. Study design and baseline characteristics of the study participants. *Diabetes*. 2002; 51:1572–1579. [PubMed: 11978658]
19. Caramori ML, Kim Y, Huang C, et al. Cellular basis of diabetic nephropathy 1. Study design and renal structural–functional relationships in patients with long-standing type 1 diabetes. *Diabetes*. 2002; 51:506–513. [PubMed: 11812762]
20. Weil EJ, Lemley KV, Mason CC, et al. Podocyte detachment and reduced glomerular capillary endothelial fenestration promote kidney disease in type 2 diabetic nephropathy. *Kidney Int*. 2012; 82:1010–1017. [PubMed: 22718189]
21. Mauer SM, Steffes MW, Ellis EN, et al. Structural-functional relationships in diabetic nephropathy. *J Clin Invest*. 1984; 74:1143–1155. [PubMed: 6480821]
22. Bulger RE, Eknayan G, Purcell DJ 2nd, et al. Endothelial characteristics of glomerular capillaries in normal, mercuric chloride-induced, and gentamicin-induced acute renal failure in the rat. *J Clin Invest*. 1983; 72:128–141. [PubMed: 6223941]
23. Fogo AB, Kon V. The glomerulus--a view from the inside--the endothelial cell. *Int J Biochem Cell Biol*. 2010; 42:1388–1397. [PubMed: 20541032]
24. Deckert T, Feldt-Rasmussen B, Borch-Johnsen K, et al. Albuminuria reflects widespread vascular damage. The Steno hypothesis. *Diabetologia*. 1989; 32:219–226. [PubMed: 2668076]
25. Lemley KV, Blouch K, Abdullah I, et al. Glomerular permselectivity at the onset of nephropathy in type 2 diabetes mellitus. *J Am Soc Nephrol*. 2000; 11:2095–2105. [PubMed: 11053486]
26. Satchell SC, Braet F. Glomerular endothelial cell fenestrations: an integral component of the glomerular filtration barrier. *Am J Physiol Renal Physiol*. 2009; 296:F947–F956. [PubMed: 19129259]
27. Fioretto P, Steffes MW, Mauer M. Glomerular structure in nonproteinuric IDDM patients with various levels of albuminuria. *Diabetes*. 1994; 43:1358–1364. [PubMed: 7926312]
28. Lopes-Virella MF, Baker NL, Hunt KJ, et al. DCCT/EDIC Research Group. Baseline markers of inflammation are associated with progression to macroalbuminuria in type 1 diabetic subjects. *Diabetes Care*. 2013; 36:2317–2323. [PubMed: 23514730]
29. Nelson RG, Newman JM, Knowler WC, et al. Incidence of end-stage renal disease in type 2 (non-insulin-dependent) diabetes mellitus in Pima Indians. *Diabetologia*. 1988; 31:730–736. [PubMed: 3240833]

30. Vasquez B, Flock EV, Savage PJ, et al. Sustained reduction of proteinuria in type 2 (non-insulin-dependent) diabetes following diet-induced reduction of hyperglycemia. *Diabetologia*. 1984; 26:127–133. [PubMed: 6714534]
31. Chasson AL, Grady HJ, Stanley MA. Determination of creatinine by means of automatic chemical analysis. *Tech Bull Regist Med Technol*. 1960; 30:207–212. [PubMed: 13692569]
32. Myers BD, Nelson RG, Tan M, et al. Progression of overt nephropathy in non-insulin-dependent diabetes. *Kidney Int*. 1995; 47:1781–1789. [PubMed: 7543961]
33. Weibel, ER. *Practical Methods for Biological Morphometry*. New York: Academic Press; 1979.
34. Pagtalunan ME, Miller PL, Jumping-Eagle S, et al. Podocyte loss and progressive glomerular injury in type II diabetes. *J Clin Invest*. 1997; 99:342–348. [PubMed: 9006003]
35. Tan JC, Busque S, Workeneh B, et al. Effects of aging on glomerular function and number in living kidney donors. *Kidney Int*. 2010; 78:686–692. [PubMed: 20463656]

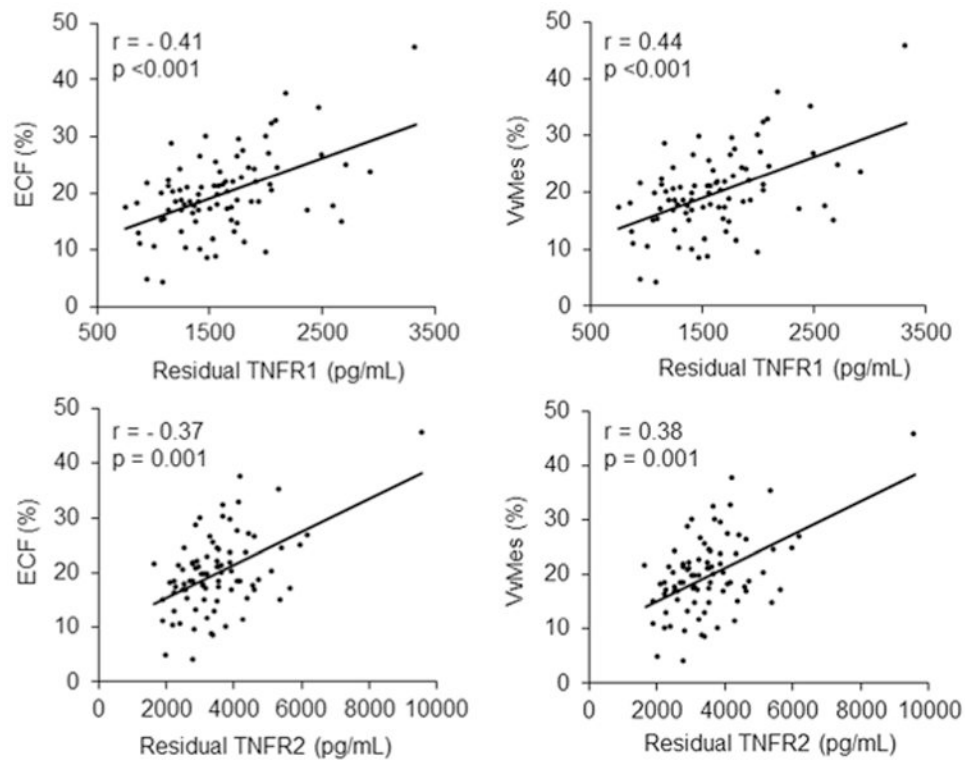


Figure 1.

Partial regression residual plot of the associations between TNFRs, percentage of normally fenestrated endothelium (ECF), and mesangial fractional volume (VvMes). The residuals were computed from regressing each of these variables on age, sex, diabetes duration, A1c, ACR, mGFR, body mass index, and mean arterial pressure. Exclusion of the single outlier did not change the significance of the associations with the two morphometric variables.

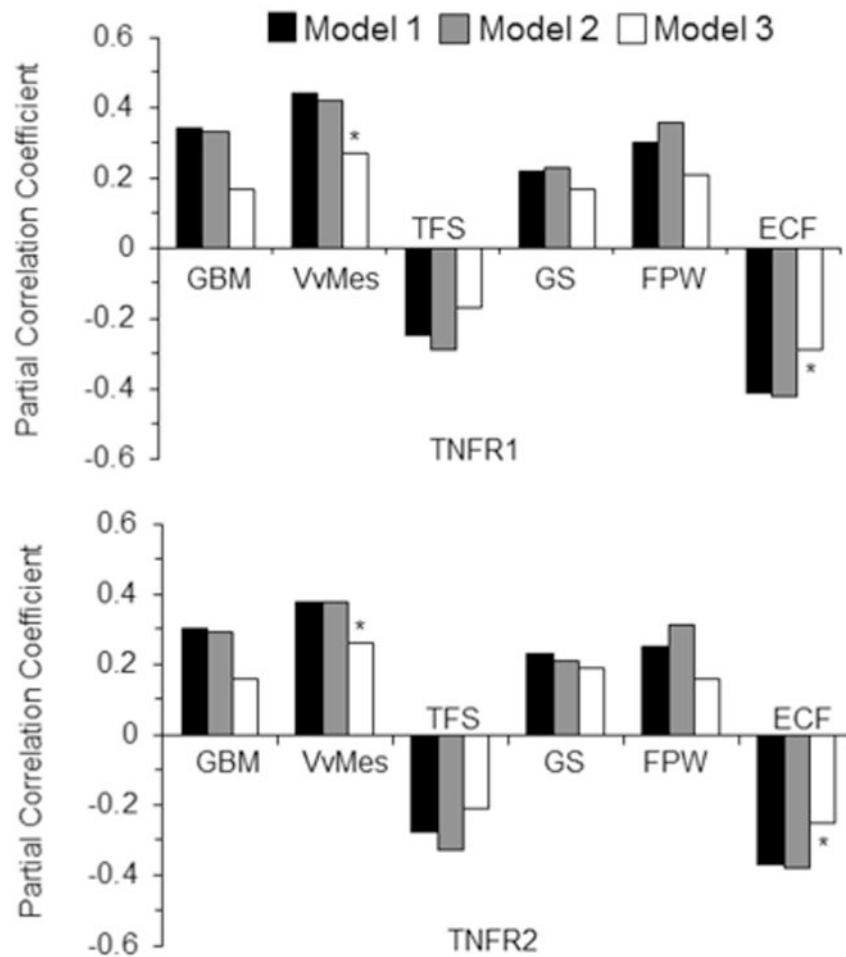


Figure 2.

Adjusted correlation coefficients for the associations between TNFRs and renal morphometric variables. Model 1 was adjusted for age, sex, diabetes duration, A1c, body mass index, and mean arterial pressure; Model 2 included Model 1 covariates and treatment assignment from the clinical trial; Model 3 included Model 1 covariates and GFR and ACR. ACR, albumin-to-creatinine ratio; BMI, body mass index; ECF, percentage of normally fenestrated endothelium; FPW, podocyte foot process width; GBM, glomerular basement membrane width; GFR, measured glomerular filtration rate; GS, percent global glomerular sclerosis; MAP, mean arterial pressure; TFS, total filtration surface per glomerulus; TNFR, tumor necrosis factor receptor; VvMes, mesangial fractional volume. All correlation coefficients for Models 1 and 2 are significant; * significant associations for Model 3.

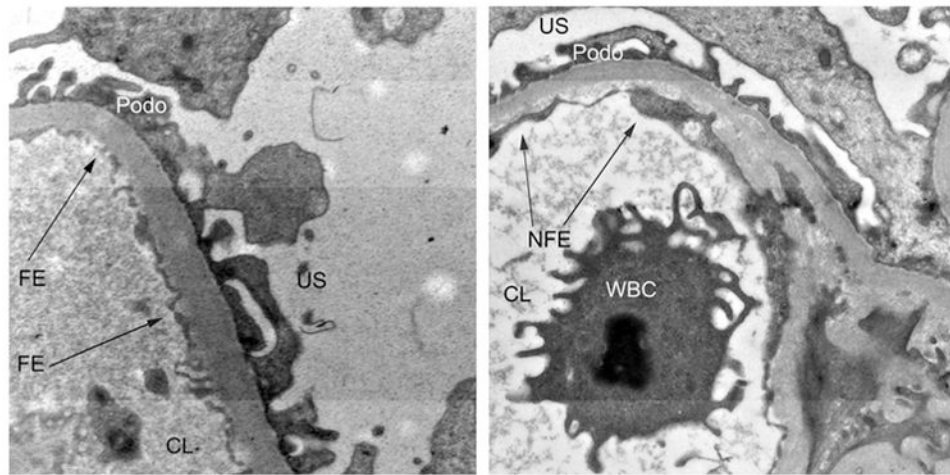


Figure 3.

Peripheral glomerular capillaries from participants with type 2 diabetes. Arrows point to capillary endothelium with normal cell fenestrations in a participant with mGFR=189 ml/min and ACR=13 mg/g (left panel) and with reduced fenestrations in a participant with mGFR=73 ml/min and ACR=1031 mg/g (right panel). Transmission electron microscopy, $\times 11,280$. CL, capillary lumen; FE, fenestrated endothelium; NFE, non-fenestrated endothelium; Podo, podocyte; US, urinary space; WBC, white blood cell.

Table 1

Characteristics of 83 participants with type 2 diabetes.

Clinical Characteristics	
Measured Markers	
TNFR1 (pg/mL)	1500 (1205-1960)
TNFR2 (pg/mL)	3283 (2670-4151)
TNF α (pg/mL) ^a	4.1 (2.9-5.7)
Other Characteristics	
Age (years) ^b	46.3 \pm 10.1
Diabetes duration (years)	14.1 (11.7-20.3)
Body mass index (kg/m ²)	34.2 (29.7-40.0)
A1c (%)	9.2 (7.6-11.2)
Systolic blood pressure (mmHg)	124 (111-132)
Diastolic blood pressure (mmHg)	77 (70-84)
Mean arterial pressure (mmHg)	93 (85-99)
Serum creatinine (mg/dL)	0.67 (0.61-0.80)
ACR (mg/g)	26 (12-127)
mGFR (ml/min)	130 (107-174)
mGFR (ml/min/1.73m ²)	119 (94-155)
Hypertension treatment (%) ^c	45
Diabetes treatment (%)	90
Lipid lowering treatment (%)	30

Values are medians (25th and 75th centile).

^a
n=74

^b
Mean \pm standard deviation

^c
n=30 on ACE and 6 on ARB.

ACR, urinary albumin-to-creatinine ratio; A1c, hemoglobin A1c; mGFR, iothalamate glomerular filtration rate; TNF α , tumor necrosis factor α ; TNFR, tumor necrosis factor receptor.

Table 2

Characteristics of 83 participants with type 2 diabetes by TNFR1 and TNFR2 percentiles.

Strata of TNFR1 (percentiles)				
Clinical Characteristics	T1 (n=19)	T2 (n=43)	T3 (n=21)	p
Measured Markers				
TNFR1 (pg/mL)	1055 (931-1120)	1500 (1374-1653)	2156 (2039-2564)	
TNFR2 (pg/mL)	2214 (1937-2471)	3284 (2935-3629)	4756 (4135-5250)	<0.001
TNFα (pg/mL) ^d	4.6 (3.1-5.9)	4.1 (2.7-5.1)	4.1 (3.0-5.6)	0.82
Other Characteristics				
Age (years) ^b	42.9 ± 9.8	46.4 ± 9.9	49.1 ± 10.3	0.15
Diabetes duration (years)	13.0 (11.2-19.4)	14.0 (11.1-20.5)	15.0 (12.4-22.4)	0.33
Body mass index (kg/m ²)	31.4 (27.6-38.4)	33.3 (29.8-40.0)	36.1 (31.7-44.6)	0.14
A1c (%)	9.4 (7.6-10.9)	9.2 (7.5-11.5)	9.2 (7.6-11.2)	0.98
Systolic blood pressure (mmHg)	119 (107-125)	124 (109-132)	127 (117-141)	0.10
Diastolic blood pressure (mmHg)	74 (70-84)	76 (70-83)	82 (72-87)	0.24
Mean arterial pressure (mmHg)	89 (83-98)	92 (85-97)	96 (88-104)	0.11
Serum creatinine (mg/dL)	0.61(0.5-0.67)	0.67 (0.60-0.79)	0.76 (0.68-0.97)	<0.001
ACR (mg/g)	18 (12-41)	26 (10-88)	97 (28-923)	0.02
mGFR (ml/min)	150 (117-170)	136 (107-188)	115 (85-133)	0.04
mGFR (ml/min/1.73m ²)	136 (117-160)	123 (94-161)	102 (81-110)	0.004
Hypertension treatment (%) ^c	26	47	57	0.14 ^d
Losartan treatment assignment (%) ^e	63	58	33	0.11 ^d
Diabetes treatment (%)	89	86	100	0.21 ^d
Lipid lowering treatment (%)	21	33	33	0.62 ^d
Strata of TNFR2 (percentiles)				
Clinical Characteristics	T1 (n=21)	T2 (n=42)	T3 (n=20)	p
Measured Markers				
TNFR1 (pg/mL)	1093 (970-1159)	1509 (1369-1854)	2218 (1981-2585)	<0.001
TNFR2 (pg/mL)	2227 (2054-2496)	3290 (2984-3590)	4848 (4561-5390)	
TNFα (pg/mL) ^f	4.6 (3.1-5.9)	3.7 (2.9-4.7)	5.0 (2.5-6.7)	0.34
Other Characteristics				
Age (years) ^b	45.3 ± 9.2	46.9 ± 10.5	48.0 ± 10.6	0.68
Diabetes duration (years)	12.9 (11.3-15.0)	14.3 (11.7-20.9)	15.3 (12.6-19.6)	0.41
Body mass index (kg/m ²)	31.4 (28.3-37.7)	33.3 (29.8-39.2)	38.6 (32.1-44.8)	0.09
A1c (%)	9.1 (6.5-10.4)	9.7 (8.3-11.5)	8.8 (7.2-11.4)	0.11
Systolic blood pressure (mmHg)	111 (107-125)	124 (114-132)	126 (117-141)	0.046
Diastolic blood pressure (mmHg)	74 (70-83)	77 (72-83)	79 (70-87)	0.56
Mean arterial pressure (mmHg)	89 (82-95)	93 (87-98)	94 (87-103)	0.17

Strata of TNFR1 (percentiles)				
Clinical Characteristics	T1 (n=19)	T2 (n=43)	T3 (n=21)	p
Serum creatinine (mg/dL)	0.65 (0.57-0.69)	0.67 (0.60-0.74)	0.8 (0.66-0.99)	0.009
ACR (mg/g)	18 (12-45)	27 (12-97)	93 (20-978)	0.03
mGFR (ml/min)	142 (126-167)	134 (107-191)	119 (102-141)	0.14
mGFR (ml/min/1.73m ²)	134 (117-158)	122 (101-169)	103 (84-121)	0.05
Hypertension treatment (%) ^g	29	48	55	0.20 ^d
Losartan treatment assignment (%) ^e	67	50	45	0.33 ^d
Diabetes treatment (%)	81	95	90	0.20 ^d
Lipid lowering treatment (%)	24	36	25	0.53 ^d

T1, TNFR1 and TNFR2 <25th percentile; T2, TNFR1 and TNFR2 25th to 75th percentile; T3, TNFR1 and TNFR2 >75th percentile.

TNFR1 values for the 25th percentile cut point=1205 pg/mL, and for the 75th percentile cut point=1960 pg/mL.

TNFR2 values for the 25th percentile cut point=2671 pg/mL, and for the 75th percentile cut point=4152 pg/mL.

Values are medians (25th and 75th centile).

^a n=74; 17; 37; 20.

^b Mean ± standard deviation.

^c T1=2 on ACE and 2 on ARB; T2=17 on ACE and 3 on ARB; T3=11 on ACE and 1 on ARB.

^d P values from Mantel-Haenszel χ^2 test for general association.

^e Assigned to receive losartan during clinical trial.

^f n=74; 19; 36; 19.

^g T1=4 on ACE and 1 on ARB; T2=17 on ACE and 3 on ARB; T3=9 on ACE and 2 on ARB.

ACR, urinary albumin-to-creatinine ratio; A1c, hemoglobin A1c; mGFR, iothalamate glomerular filtration rate; TNF α , tumor necrosis factor α ; TNFR, tumor necrosis factor receptor.

Table 3

Renal glomerular structural characteristics of 83 participants with type 2 diabetes by TNFR1 percentiles.

General Structural Variables	Strata of TNFR1 (percentiles)			p
	T1 (n=19)	T2 (n=43)	T3 (n=21)	
Mean glomerular volume ($\times 10^6 \mu^3$)	5.5 (4.7-6.4)	5.8 (4.9-6.9)	5.4 (4.2-7.4)	0.46
Glomerular basement membrane width (nm)	513 (367-560)	457 (393-611)	561 (474-649)	0.02
Cortical interstitial fractional volume (%)	30 (28-33)	29 (24-33)	31 (29-35)	0.18
Mesangial fractional volume (%)	17 (13-20)	17 (13-23)	27 (18-32)	0.002
Total filtration surface per glomerulus ($\times 10^5 \mu^2$)	4.2 (3.4-5.1)	4.2 (3.5-5.6)	3.3 (2.3-4.0)	0.02
Non-podocyte number per glomerulus	3171 (2066-4447)	3696 (2705-4803)	4010 (2991-5377)	0.06
Global glomerular sclerosis (%)	3.3 (0-16.4)	5.4 (0-17.7)	10.2 (0-32.1)	0.30
Podocyte structural variables				
Podocyte number per glomerulus	672 (518-724)	610 (462-733)	525 (413-725)	0.46
Filtration slit frequency (slits/mm)	1489 (1266-1648)	1354 (1231-1518)	1296 (1165-1485)	0.08
Foot process width (nm)	409 (378-499)	470 (415-525)	494 (430-570)	0.02
Podocyte detachment (%)	0.28 (0-0.92)	0.46 (0-1.07)	0.56 (0-2.26)	0.83
Endothelial variable				
Fenestrated endothelium (%)	31.2 (25.9-37.8)	29.0 (22.3-33.0)	22.9 (17.6-24.5)	<0.001

General Structural Variables	Strata of TNFR2 (percentiles)			p
	T1 (n=21)	T2 (n=42)	T3 (n=20)	
Mean glomerular volume ($\times 10^6 \mu^3$)	5.5 (5.0-6.7)	5.7 (4.5-6.8)	5.5 (4.3-7.3)	0.99
Glomerular basement membrane width (nm)	455 (367-556)	503 (411-588)	563 (448-661)	0.06
Cortical interstitial fractional volume (%)	29 (25-33)	30 (24-35)	30 (26-33)	0.87
Mesangial fractional volume (%)	15 (12-19)	20 (14-26)	22 (16-27)	0.01
Total filtration surface per glomerulus ($\times 10^5 \mu^2$)	4.5 (3.7-5.7)	4.2 (3.4-5.1)	3.4 (2.6-4.0)	0.03
Non-podocyte number per glomerulus	3171 (2360-4447)	3752 (2922-4982)	3863 (2760-4719)	0.32
Global glomerular sclerosis (%)	3.3 (0-13.0)	4.8 (0-16.5)	15.1 (0-32)	0.17
Podocyte structural variables				
Podocyte number per glomerulus	672 (538-733)	604 (462-724)	551 (374-705)	0.21
Filtration slit frequency (slits/mm)	1489 (1290-1621)	1350 (1237-1490)	1252 (1145-1523)	0.03
Foot process width (nm)	415 (388-449)	481 (428-524)	513 (404-571)	0.02
Podocyte detachment (%)	0.10 (0-0.77)	0.39 (0-1.16)	0.55 (0-1.8)	0.64
Endothelial variable				
Fenestrated endothelium (%)	32.8 (27.6-36.1)	26.8 (22.15-31.74)	23.4 (18.5- 26.6)	<0.001

T1, TNFR1 and TNFR2 <25th percentile; T2, TNFR1 and TNFR2 25th to 75th percentile; T3, TNFR1 and TNFR2 >75th percentile.TNFR1 values for the 25th percentile cut point=1205 pg/mL, and for the 75th percentile cut point=1960 pg/mL.TNFR2 values for the 25th percentile cut point=2671 pg/mL, and for the 75th percentile cut point=4152 pg/mL.Values are medians (25th and 75th centile).

Spearman's correlations of TNF α and TNFRs with clinical characteristics and glomerular structural parameters in 83 Pima Indians with type 2 diabetes mellitus.

Table 4

	TNFR1		TNFR2		TNF α	
	r	p	r	p	r	p
Clinical variables						
Age (years)	0.29	0.01	0.13	0.26	-0.12	0.30
Diabetes duration (years)	0.24	0.03	0.20	0.07	-0.17	0.14
Mean arterial pressure (mmHg)	0.15	0.16	0.21	0.06	0.23	0.04
Body mass index (kg/m ²)	0.23	0.03	0.28	0.01	-0.05	0.67
A1c (%)	-0.13	0.25	0.05	0.66	0.27	0.02
mGFR (ml/min)	-0.35	0.001	-0.28	0.01	0.13	0.28
ACR (mg/g)	0.36	0.001	0.37	0.001	0.03	0.79
Measured markers						
TNFR1	1		0.84	<0.001	-0.14	0.23
TNFR2	0.84	<0.001	1		-0.05	0.69
TNF α (pg/mL)	-0.14	0.23	-0.05	0.69	1	
General structural variables						
Mean glomerular volume ($\times 10^6 \mu^3$)	-0.03	0.80	0.005	0.96	0.17	0.14
Glomerular basement membrane width (nm)	0.23	0.04	0.26	0.02	0.01	0.91
Cortical interstitial fractional volume (%)	0.12	0.28	0.07	0.54	0.10	0.38
Mesangial fractional volume (%)	0.36	0.001	0.38	<0.001	-0.01	0.91
Total filtration surface per glomerulus ($\times 10^5 \mu^2$)	-0.27	0.01	-0.29	0.01	-0.05	0.68
Non-podocyte number per glomerulus	0.18	0.10	0.18	0.10	0.12	0.31
Global glomerular sclerosis (%)	0.25	0.02	0.18	0.11	-0.19	0.11
Podocyte structural variables						
Podocyte number per glomerulus	-0.23	0.04	-0.16	0.14	0.15	0.20
Filtration slit frequency (slits/mm)	-0.24	0.03	-0.29	0.01	-0.17	0.14
Foot process width (nm)	0.29	0.01	0.31	0.004	0.15	0.20
Podocyte detachment (%)	0.05	0.63	0.08	0.45	-0.13	0.27

Endothelial variable	TNFR1		TNFR2		TNFα	
	r	p	r	p	r	p
Fenestrated endothelium (%)	-0.42	<0.001	-0.43	<0.001	0.04	0.75

ACR, urinary albumin-to-creatinine ratio; A1c, hemoglobin A1c; mGFR, iothalamate glomerular filtration rate; r, correlation coefficient; TNFα, tumor necrosis factor α; TNFR, tumor necrosis factor receptor.